IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Ferdinand Hermann BAHLMANN, *et al.*
Serial No.: 10/522,426
Filed: March 25, 2005
For: USE OF ERYTHROPOIETIN

Confirmation No.: 5804
Group Art Unit: 1654
Examiner: Thomas S. Heard

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SECOND DECLARATION OF PROF. DR. HERMANN HALLER UNDER 37 C.F.R. §1.132

Sir:

1. I, Hermann Haller, am a German citizen, residing at An der Trift 8D, 30559 Hannover, Germany. I am a trained Physician and Scientist, working in the field of kidney disease at the university level for several years. I am the same Hermann Haller who previously submitted a first declaration in this application that was executed on 2 of May 2008.

2. As indicated in my previous declaration, I am a co-inventor of this application, i.e., Serial No. 10/522,426. I have reviewed the Office Action from the United States Patent and Trademark Office dated June 23, 2008 and I understand the rejections set forth therein. In addition, as stated in my previous declaration, I am familiar with the references cited as a basis for the various grounds of rejection set forth in the Office Action. I am making this declaration in support of the claims of application Serial No. 10/522,426

3. After injury to the skin, repair mechanisms begin immediately to restore the skin's barrier function. A variety of different regenerative processes, as described below, take place in parallel in order to close the wound as quickly as possible. The goal of these processes is to maintain the skin's protective function. That is, the homeostasis of the body of one with such an injury to the skin can only be maintained if the skin prevents infections caused due to the passage of germs through lesions in the skin.

4. Wound healing can be divided into three phases: (A) an Inflammatory Phase; (B) a Granulation Phase; and (C) a Scar Remodeling Phase. The Inflammatory Phase comprises Phase 1, which involves necrosis and skin ulceration (see Exhibit 1). The Granulation Phase may be separated into two phases, i.e., Phase 2 wherein the wound displays numerous areas of granulative and resorptive inflammation and wherein re-epithilization is beginning to occur (Exhibit 2), and Phase 3 displaying a moderate level of cell-rich granulative inflammation, wherein re-epithillization is progressing (as shown in Exhibit 3). The next phase, i.e., the scar remodeling phase, can be divided into three phases, nos 4-6. Phase 4 involves formation of granulative tissue having a low cell density and which exhibits complete re-epithilization (Exhibit 4). Phase 5 results in the production of a cell-rich scar (Exhibit 5). Finally, in Phase 6 a scar with a low cell density is produced (Exhibit 6).

5. This declaration sets forth the results of a series of experiments, carried out by me or under my direction and control, in which the effect(s) of Erythropoietin on the three phases of wound healing was studied. We chose for these experiments an animal model of diabetes. Skin lesions in diabetes are a significant health problem world-wide and represent a major therapeutical challenge We and others have previously demonstrated that skin lesions in this model are reproducible and useful for testing novel therapeutic tools in wound healing. As a result of these experiments, which are detailed further below, it was found that the administration of erythropoietin (hereinafter "EPO") serves to accelerate the granulation phase of wound healing. This is a major precondition for primary wound healing. Accelerated granulation decreases the rate of wound infections, is associated with angiogenesis and reduces complications of wound healing. While the granulation phase was significantly enhanced no statistically relevant effect on the scar remodeling phase was detected based upon the results of these experiments. This difference underlines the specific effect of EPO on cellular mechanisms of wound healing. Furthermore, the primary finding obtained on the basis of the experiments discussed hereinbelow was that, in contrast to the high dosis level of EPO established, i.e., in the prior art references cited by the U.S. Examiner to reject the claims of the

present application, a systemic low dose application of such EPO (i.e., at 0.1 μg/kg/week) which is within the dosage level set forth in the claims of the application, serves to unexpectedly accelerate the Granulation Phase of wound healing.

6. The experiments referred to herein were carried out using groups of 10-12 week old male rats. Each group constituted 38 rats, wherein the rats were treated and then thereafter underwent analysis, as described below. The local Ethics Committee approved the animal treatment protocol. The wounds were placed on the backs of the animals, on both sides of the spine. Under anesthesia (i.e., Ketamine @ 9mg/100g body weight with ROMPUN® @ 0.2 mg/100g body weight), wounding areas measuring 6mm in circumference were applied to the animals. The animals received either NaCl (control/placebo) or 0.1 μg/kg (low dose) or 0.5 μg/kg (high dose) ARANESP®. The ARANESP® was given intravenously immediately after wounding, and then again one week thereafter. Wound healing was assessed by planimetry and laser-doppler analysis. The data from these experiments is displayed in the appended Exhibits as mean values and SEM. An unpaired t-test was used to test for significance, whereupon a p-value of < 0.05 was considered to be significant.

7. The bar graph provided in Fig. A (Exhibit 7) demonstrates unexpectedly improved results in wound healing obtained during the Granulation Phase at day 3 and day 6 produced following administration of EPO at dosage levels as recited in the claims of our present application, versus results achieved with the use of a higher dosage as taught, for example, in the prior art cited to reject the present claims of our application. The comparison is among four (4) groups of rats, i.e., non-diabetic rats receiving a placebo (NaCl Control); diabetic rats receiving a placebo (NaCl Control); diabetic rats receiving a low dose (0.1 μg/kg body weight, i.e., according to the claims) of ARANESP®; and diabetic rats receiving a 'standard', i.e., "high" dose (0.5 μg/kg body weight) of ARANESP®, i.e., according to the teachings of the prior art. The degree of wound closure is indicated along the left axis of the graph. The results clearly demonstrate that the systemic low dosage application of EPO, as recited in the presently pending claims, significantly accelerates the Granulation Phase of the wound healing process.

8. A significant delay in wound healing, i.e., during the Granulation Phase, was also found to occur in diabetic animals compared to the non-diabetic placebo-receiving group of rats at day 3 after surgical wounding. However, the degree of wound healing in the diabetic rats was found to be improved by an EPO treatment. An impressive improvement was obtained with the application of low-dose EPO; however, in contrast, a significantly lower degree of improvement was found to occur in the case of the high-dose EPO group of animals at day 3. The above-described improvement is displayed in Fig. B found in Exhibit 8 attached to this declaration. As indicated therein, Fig. B illustrates the relative progress of wound healing obtained in the different treatment groups at postoperative day 3. The healing process was normalized to the level of the non-diabetic animals.

9. Furthermore, as shown in Fig. C (Exhibit 9) at Day 6 the wound healing in the low-dose EPO group (0.1/μg/kg/week) shows no significant difference between the non-diabetic animals (Group 1) and the diabetic animals (Group 3) after low-dose EPO treatment. The healing process also was normalized to the level of the non-diabetic animals. Additionally, the high-dose EPO group (Group 4) shows no significant difference when compared to the non-treated diabetic animals (Group 2) which received the placebo.

10. The improved results achieved by the method recited in the pending application, as demonstrated in, e.g., Exhibits 7-9 to this declaration demonstrate the superiority of low-dose EPO treatment, as presently claimed, in comparison to the high-dose treatment (as taught for use, e.g., in the references cited by the Examiner to reject the present claims), that is achievable in diabetic patients suffering from problems in wound healing.

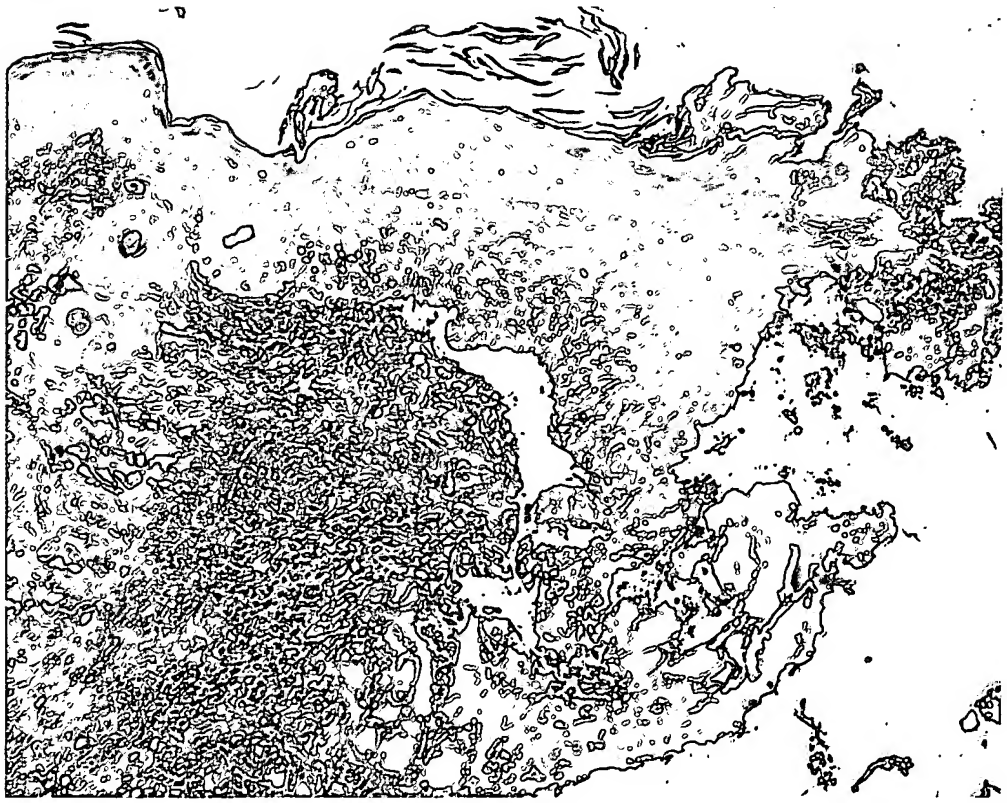
11. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 16/12/08

BY: _____
Hermann Haller

EXHIBIT 1

Phase I



Necrosis/Skin ulceration

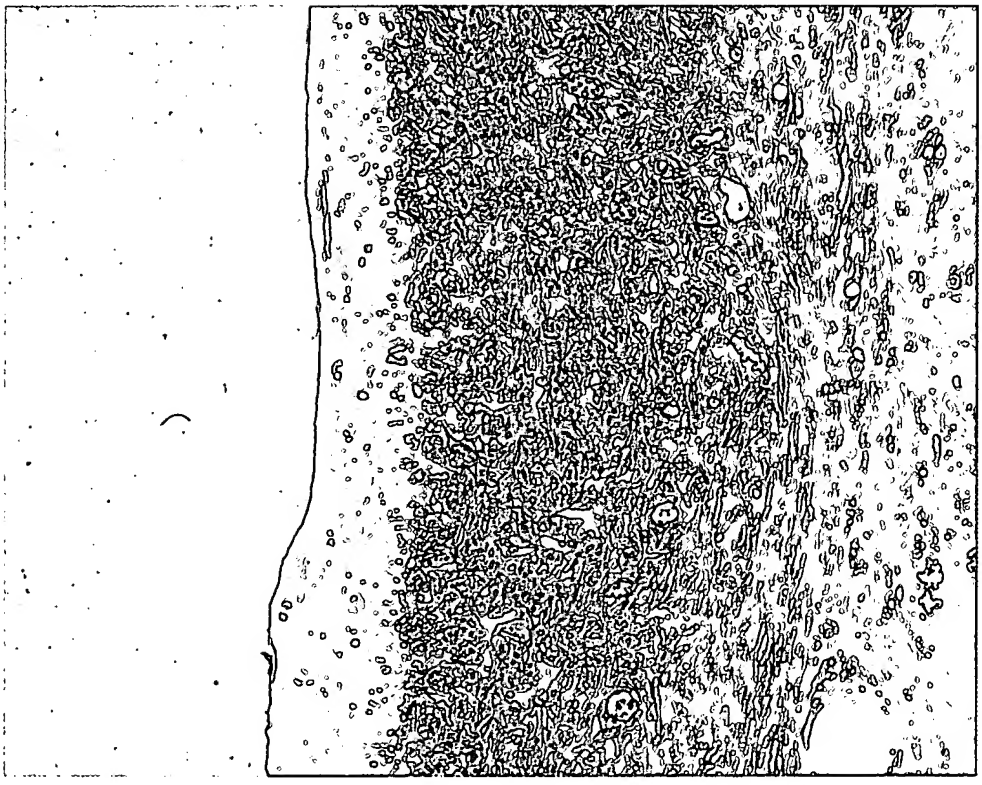
EXHIBIT 2

Phase II

Numerous granulative and resorptive Inflammation wherein reepitheliasation is beginning

EXHIBIT 3

Phase III



Moderate cell-rich granulative inflammation wherein reepitheliasation is progressing

EXHIBIT 4

Phase IV

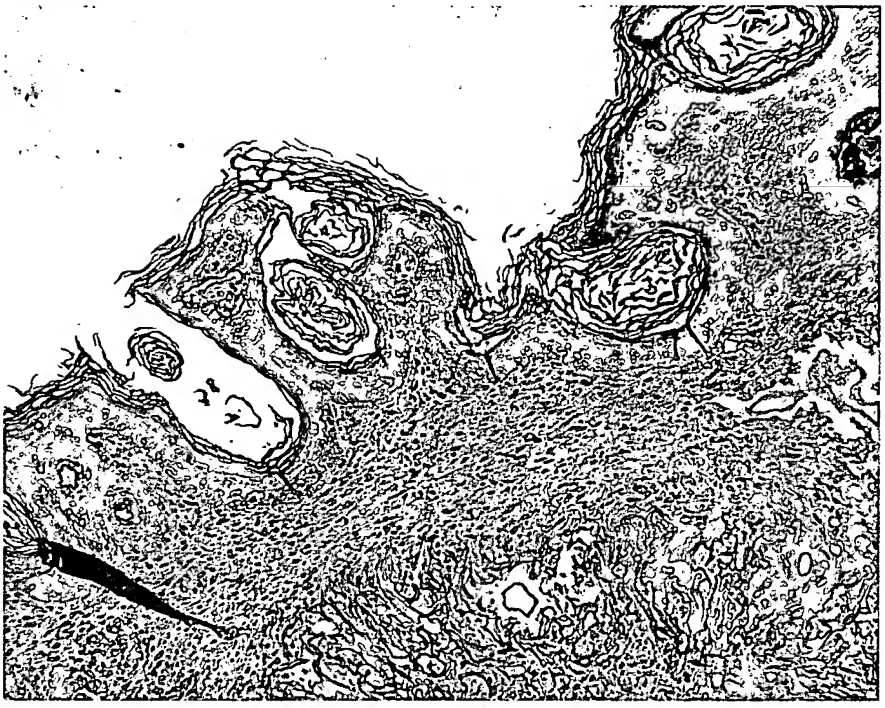


EXHIBIT 5

Phase V

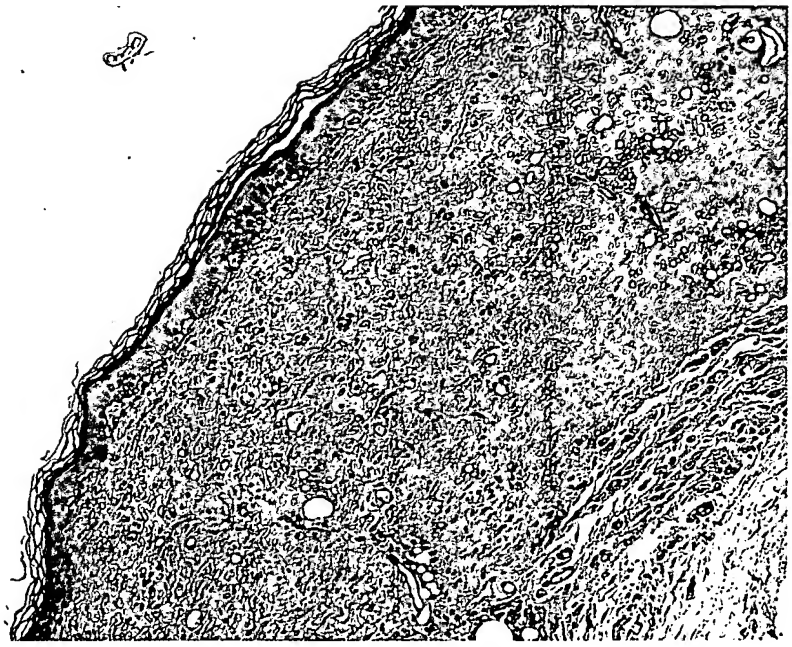


EXHIBIT 6

Phase V with vascular proliferation

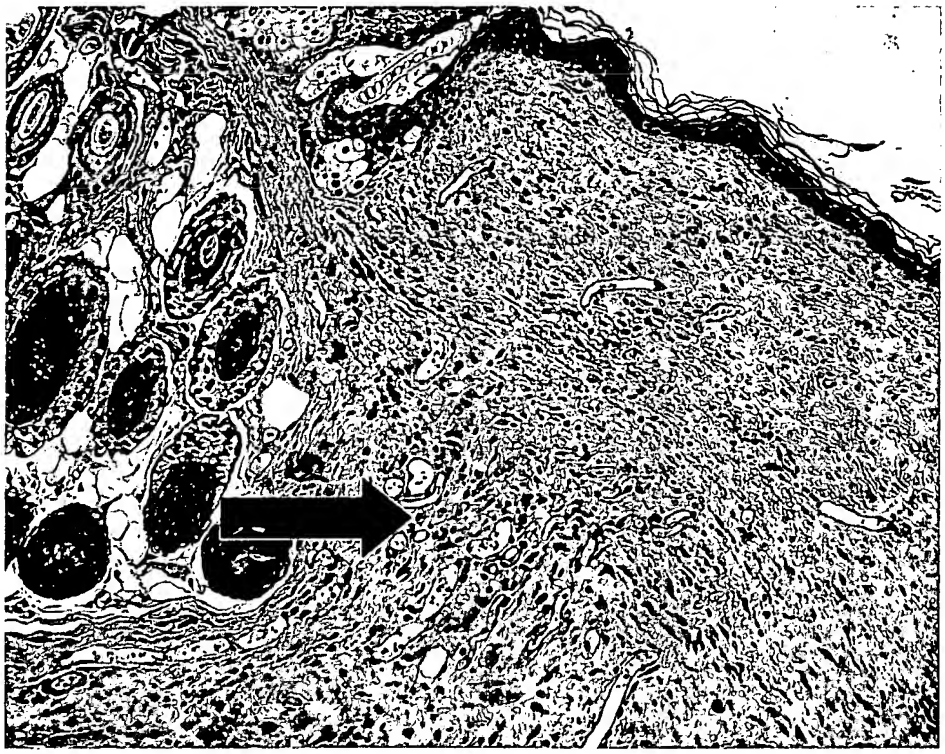


EXHIBIT 7

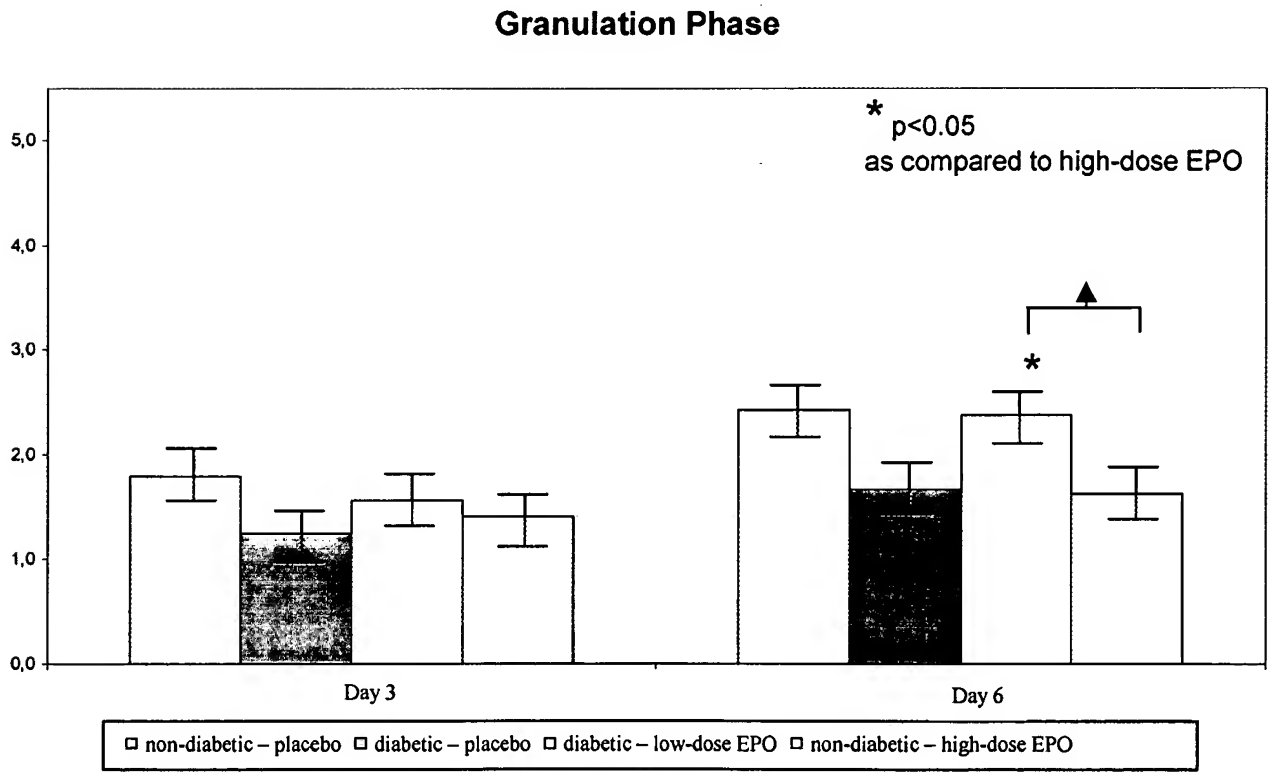


Fig. A: Process of wound healing in the different treatment groups during Granulation Phase at day 3 and 6

EXHIBIT 8

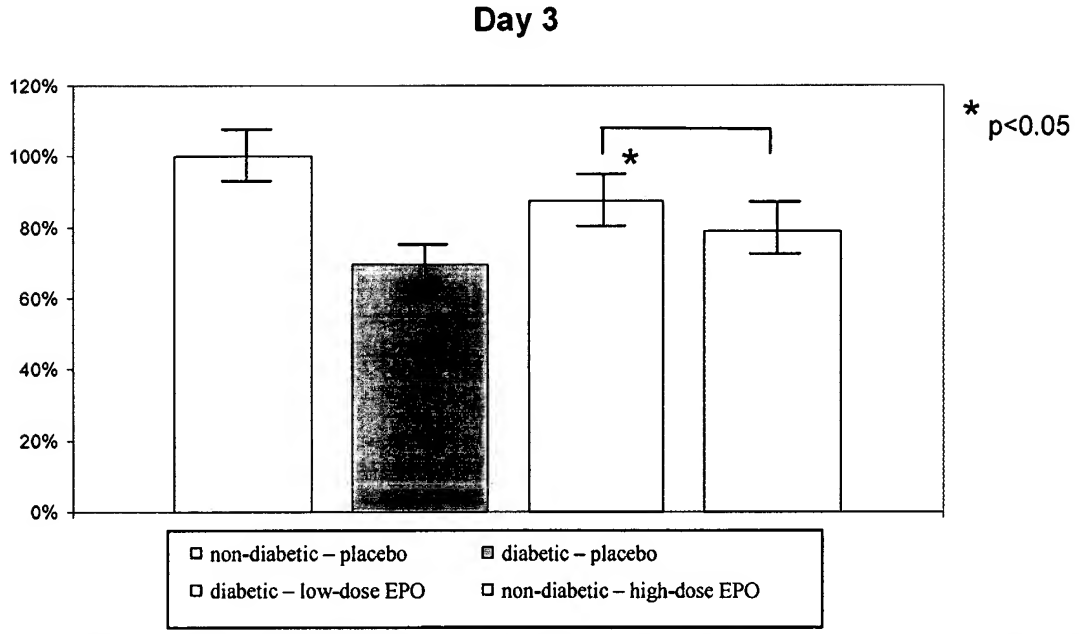


Fig. B: Relative process of wound healing in the different treatment groups at postoperative day 3. The healing process was normalised to the level of non diabetic animals.

EXHIBIT 9

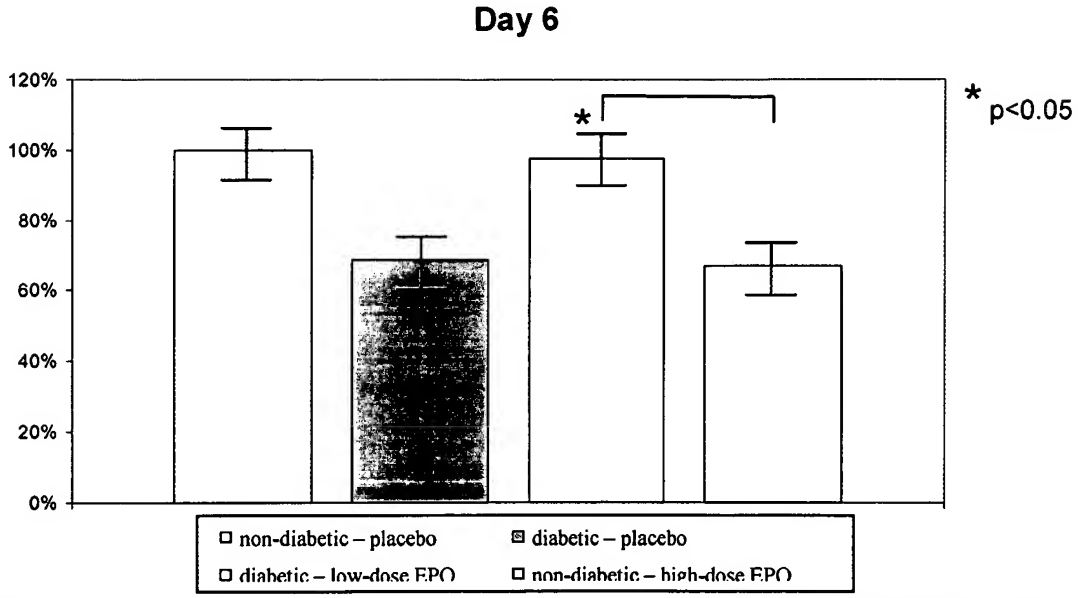


Fig. C: Relative process of wound healing in the different treatment groups at postoperative day 6. The healing process was normalised to the level of non diabetic animals.